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PATENT
Docket No. 01819232

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
Girish V. Shah

SERIAL NUMBER: 09/251,133

FILED: FEBRUARY 16, 1999

TITLE: NEUROENDOCRINE MARKER OF PROSTATE CANCER AND METHOD FOR PRODUCING SAME

GROUP ART UNIT: 1642

EXAMINER: C. Yaen

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DECLARATION OF GIRISH SHAH, PH.D. UNDER 37 C.F.R. §1.132

Assistant Commissioner of Patents
Washington, D. C. 20231

Dear Sir:

I, Girish V. Shah, Ph.D., declare and state as follows:

1. I am the sole inventor of the above-captioned patent application. I have personal knowledge of the facts stated herein.

2. I have reviewed the Office Action mailed by the Patent and Trademark Office on May 21, 2002 in this case and submit this Declaration to support my position.

3. My patent application discloses and claims, among other things, an isolated neuroendocrine marker ("NEM") polypeptide useful in the treatment of cancer and isolated DNA encoding the polypeptide.

4. In 1995, I unexpectedly isolated the cDNA encoding the novel NEM polypeptide disclosed in the present application while trying to clone a cDNA encoding a different neuroendocrine peptide. At the time, I assumed that the newly obtained cDNA sequence may also belong to cDNAs encoding the neuroendocrine family of peptides so I performed in-situ hybridization to determine its expression. In-situ hybridization studies using the novel NEM nucleotide sequence on prostate cancer tissue sections revealed that NEM was selectively expressed in prostate cancer cells.

5. I originally sequenced this novel NEM cDNA and obtained the cDNA sequence of SEQ ID NO:2 and the amino acid sequence of SEQ ID NO:1 deduced from SEQ ID NO:2. However, as discussed below, later examination showed that the sequencing was sub par and that sequencing errors related to machine error were introduced into SEQ ID NO:2 and consequently SEQ ID NO:1. As a result, both SEQ ID NO:2 and SEQ ID NO:1 include errors.

6. Since the in-situ hybridization results provided an interesting observation, I expressed the novel isolated cDNA in a standard eukaryotic expression vector containing a histidine affinity purification tag, for ease of purification, in a human cell line and purified the NEM peptide that was produced by affinity chromatography. The purified peptide was used for studying stimulation of proliferation and invasion, the protocols and results of which are included in my application. The peptide was then injected into a rabbit to obtain antisera. This antisera was used to demonstrate the inhibition of proliferation and invasion of prostate cancer cells and immunohistochemistry results.

7. In November 1998, subsequent to these studies and after the filing of U.S. Provisional Application No. 60/074,809, filed February 17, 1998, I re-sequenced the original

isolated cDNA sample at a different facility and discovered minor sequencing errors in SEQ ID NO:2. Some of the cloning vector sequence was also inadvertently included at the 5' and 3' end of SEQ ID NO:2, which was merely a copying error. Due to gaps in the 3'-untranslated region of SEQ ID NO:2 (indicated by "S" and "N"), it would be clear to a scientist in this field that SEQ ID NO:2 contained sequencing errors.

8. In the process of re-sequencing, I obtained cDNA SEQ ID NO:3 and deduced the corresponding amino acid SEQ ID NO: 6. I then included SEQ ID NO:3 and SEQ ID NO:6, as well as potential sequences that could be derived by alternate translation, in my present utility application. If the vector sequences were removed from SEQ ID NO:2 and compared to SEQ ID NO:3, the two sequences have a very high degree of homology (i.e. 92%).

9. As disclosed above, SEQ ID NO:6 was derived from cDNA SEQ ID NO:3 by deduction. The novel isolated cDNA encoding NEM was spliced to the initiator methionine codon (AUG) in an expression vector, using standard recombinant cDNA techniques, in such a way such that the first codon of the novel isolated cDNA that was expressed was AGA (Arginine) (SEQ ID NO:3, nucleotides 1-3). The vector was then transfected into a human cell line to express the novel NEM polypeptide. I then purified the expressed NEM polypeptide for use in further studies, as disclosed in my application. Due to the sequence specific manner that the novel isolated cDNA was spliced to the methionine initiator of the vector, it was clear to me, and I believe it would be clear to a scientist in this field, that the first expressed codon of the novel isolated cDNA is indeed AGA, which followed the AUG methionine in the vector. This method provides easy identification of the reading frame used for translating the cDNA to produce active NEM polypeptide. This method is widely used by molecular biologists to deduce the amino acid sequence of a polypeptide expressed from a cDNA and is an accepted practice in

the industry because it is often difficult to sequence long peptide or protein sequences with high degree of accuracy. *See* Najjar, SM & Lewis, RE, Gene 230 (1):41-45 (1999); Oeda, T et al., Molecular Endocrinology 6(8):1216-26 (1992); Machida, CA et al., Moleculari Pharmacology 41(4):652-59.

10. The polypeptide and the cDNA used in all the experiments reported in the present application were from the same original sample I obtained prior to the filing of my provisional application. There has been no change in any of the materials used, regardless of the sequence identity characterization. Since the time I identified the novel isolated cDNA through the filing of my provisional application and through the filing of my utility application, I was in possession of the novel isolated NEM cDNA characterized by SEQ ID NO:3 at all times.

11. Although I had initially assumed that SEQ ID NO:1 was the correct translational amino acid sequence of cDNA SEQ ID NO:2, subsequent sequencing and more careful analysis clearly showed that this was an error and that the correct amino acid sequence, to the best of my knowledge, is amino acid SEQ ID NO:6 derived from cDNA SEQ ID NO:3.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

10/15/02

Date

Girish V. Shah

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